ORIGINAL ARTICLE

The effect of surgical exposure of nerves and muscles in neurophysiologic tests on rats

Efeito da exposição cirúrgica de nervos e músculos no teste neurofisiológico em ratos

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ABSTRACT

The neurophysiologic study in the modality of electroneuromyography (ENMG) determined and quantified the components in the motor ambit. The main data supplied by the examination was from the motor and sensory nerve conduction studies and by electromyography. However, many factors can interfere with the nervous response to electrostimulation, such as: age, gender, temperature, humidity, and other things. The aim of this work was to verify the effect of surgical exposure of the sciatic, common fibular, tibial, and cranial tibial muscle nerves in a neurophysiologic test on rats. Twenty (20) Wistar male rats were utilized, with approximately 80 days of age, divided into two groups. In the normal group the exam was made without the surgical exposure of the fibular nerve. In the surgical group the common fibular nerve was exposed. With the experimental model utilized, it was concluded that the neurophysiologic test done on animals with nerves and muscles surgically exposed is viable, since the alteration in animal temperature did not interfere significantly with the values of the electrophysiological parameters observed. In addition, the exposure of nerves and muscles allows stimulation of an exact point on the target nerve.

Keywords: Neurophysiology, Electromyography, Sciatic Nerve/surgery, Peroneal Nerve/surgery, Rats

RESUMO

O estudo neurofisiológico, na modalidade da eletroneuromiografia (ENMG), determina e quantifica a integridade de componentes da unidade motora. Os principais dados fornecidos pelo exame eletroneuromiográfico são os estudos de condução nervosa motora, sensitiva e eletromiografia. No entanto, vários fatores podem interferir sobre a resposta nervosa à eletroestimulação, tais como: idade, sexo, temperatura, umidade e outros. O objetivo deste trabalho foi verificar o efeito da exposição cirúrgica dos nervos ciático, fibular comum, tibial e do músculo tibial cranial no teste neurofisiológico em ratos. Foram utilizados 20 ratos, Wistar, machos com aproximadamente 80 dias, divididos em dois grupos. No grupo normal o exame foi realizado sem a exposição cirúrgica do nervo fibular. No grupo cirúrgico houve a exposição do nervo fibular comum. Com o modelo experimental utilizado, concluiu-se que o teste neurofisiológico realizado em animais com nervos e músculos expostos cirurgicamente é viável, uma vez que a alteração da temperatura do animal não interferiu significativamente nos valores dos parâmetros eletrofisiológicos observados. Além disso, a exposição de nervos e músculos permite estimular um ponto exato no nervo alvo.

Palavras-chave: *Neurofisiologia, Eletromiografia, Nervo Ciático/cirurgia, Nervo Fibular/cirurgia, Ratos*

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INTRODUCTION

Traumatic lesions of the peripheral nerves with extensive functional loss are challenges to the Medicine of Rehabilitation, which almost always first go through a surgical attempt. With the development and growing practice of microsurgery for neural repair as the attempt to recover function in pronounced nerve lesions, the need for consistent and reliable models has become greater. Electro-physiological evaluation of the peripheral nerves has been a fundamental part of the experimental models to evaluate neural regeneration and specifically muscular reinnervation, as much in surgical practices as in physiotherapy.[1] The existing electro-physiological methods are adaptations of the model for examining humans, performed in accordance with the animal tests, the nerve to be studied, and the type of experiment.[1-8]

The main purpose of the electro-physiological study is to determine and quantify the function and the disturbances of the peripheral nervous system, specifically the sensory and motor nerves, the neuromuscular junction, and the motor unit.[9] The main data provided by the exam are the location, the size and physiopathologic characteristics of the lesion, and the possibilities of reinnervation.[10]

In the experimental models, the electrophysiological evaluation seeks to determine the nervous conduction through the site or sites of surgical intervention and the motor response to the treatment, quantifying them as reliably as possible. In this way the evolution of different treatments can be monitored.

Many factors such as age, gender, muscle, type of electrode, sensitivity, stimulus frequency, frequency filter, alteration in temperature, and humidity can interfere with the responses to the electro-stimulation. Thus in many works, the standardization of parameters was observed referring to the stimulator, pre-amplifier and oscilloscope, maintenance of ambient temperature, maintenance of the animal's body temperature, and the application of mineral oil to avoid the desiccation of the exposed structures.[11-14]

OBJECTIVE

The object of this work is to verify the effect of surgical exposure of the sciatic, common fibular, tibial, and fibular muscle nerves in neurophysiologic tests in rats.

METHOD

Twenty (20) Wistar male rats approximately 80 days of age were utilized in the experiment. They were conditioned in an appropriate place (24o C, 60-70% humidity) in a 12-hour cycle of light-dark. The rats were supplied by the Biotério Central da Universidade do Estado de São Paulo (UNESP) (São Paulo State University Central Biotery), in Botucatu, São Paulo state, Brazil.

The experimental protocols were approved by the Experimentação Animal da Faculdade de Medicina da Universidade do Estado de São Paulo (UNESP) (Ethics Committee in Animal Experimentation from the São Paulo State University School of Medicine), in Botucatu, São Paulo state, under the protocol number 493.

The animals were weighed and anesthetized with 3% sodium pentobarbital (30mg/Kg, i.p.) and positioned in dorsal decubitus with the right hind limb shaved. There were two experimental groups.

In the normal group, the electro-physiological test was done without surgical exposure of nerves and muscles. Between the stimulation electrode and the skin an electrolytic conductor was utilized to facilitate the electrical conduction.

After performing the test on the normal group, to form the surgical group, an incision was made in the same animal on its right pelvic limb, allowing exposure and desiccation of the cranial tibial muscle and of the sciatic, tibial, and common fibular nerves. The electrical stimulation was triggered on the sciatic nerve by a bipolar electrode, specially produced in the form of a hook with the cathode 2mm away from the anode, and positioned to isolate the sciatic nerve from the adjacent structures. The tibial nerve was sectioned to avoid possible interferences of activity from the gastrocnemius muscle.

The animals had their temperature monitored by a rectal thermometer during the neurophysiologic testing.

Neuroconduction

A Sapphire II 4ME device was utilized. The standardization of the test was made for the capture electrode (monopolar needles) and its position in the target muscle (active electrode in the central region of the muscle, the reference electrode near the muscular insertion tendon, similar to the routine of motor nerve conduction with surface electrodes to register the compound motor action potential (CMAP).[2]

The stimulation electrode was specially built (hook electrode, bipolar with 2mm distance between the cathode and the anode), the frequency of stimulation was 1 pps and the duration was 100μs with the neutral electrode (ground) located at the base of the rat's tail. The low frequency filter was kept at 0.1 Hz and the high frequency filter was kept at 100Hz. The intensity of stimulation, measured in volts, was gradually increased until the register of maximum amplitude of the CMAP obtained.

The animals were standardized as to their age (80 days old), gender (males), and muscle where the capture electrodes were inserted (right cranial tibial muscle).

After the test the animals were killed with a lethal dose of sodium pentobarbital. The values of amplitude, latency, duration, and area were compared between the groups.

Statistical Analysis

Considering the results obtained representative measurements were calculated for average and standard deviation. The paired t-test was utilized with a significance level of 5%, to compare the values of latency, amplitude, duration, area of the CMAP (s), and temperature.

RESULTS

Table 1 shows the results obtained for latency, amplitude, duration of response to the electrical stimulation, area, body temperature, and intensity of the electrical stimulation. The surgical exposure of the sciatic, tibial, common fibular nerves, and of the cranial tibial muscle among other things diminished the body temperature significantly, though it did not alter the results of the neurophysiologic test.

The intensity of the stimulation was increased gradually until the register of the

Table 1 - Results of the neurophysiologic test.

Group	Normal	Surgical
Latency (ms)	1,43 ± 0,36	1,39 ± 0,29
Amplitude (mV)	26,22 ± 7,63	27,16 ± 8,28
Area (μVs)	36,43 ± 11,37	37,34 ± 12,02
Duration (ms)	2,46 ± 0,22	2,42 ± 0,28
Temperature (ºC)	34,46 ± 0,97*	33,76 ± 1,13
Intensity of stimulation (V)	47,1 ± 9,3*	4,8 ± 0,6

* Significant difference (p<0.05).

highest amplitude was obtained. In the animal group it was necessary to utilize 47.1 (± 9.3) volts; while to obtain the maximum amplitude in the surgical group only 4.8 (± 0.6) volts was necessary.

DISCUSSION

In the study of nervous regeneration, amplitude and latency values have shown themselves as important allies to provide data about the integrity of nerves and muscles after the repair of peripheral nerves.[3,4,6,7]

The standardization of the test in relation to the choice of capture electrodes (monopolar needles) and their position in the target muscle, with the active electrode in the central region of the muscle, the reference electrode near the insertion tendon, similar to the recommendation of the "muscular venter system – tendon"[1,15] is crucial in obtaining a comparable CMAP. That does not occur when the register of the motor potential with concentric needle electrode is utilized.

This hook-style stimulation electrode used to stimulate the nerve and distance it from adjacent tissues avoids the contamination of the target CMAP by the conduction of the stimulation through nearby muscular or nerve tissues other than the nerve studied.

The authors judged that the technical standardization of the animals in relation to age (80 days old) and gender (males), and the nerve-muscle group (sciatic and right cranial tibial muscle) in this study aided in the comparison of data.

Temperature is an important factor that can interfere with the electro-physiological test, and the exposure of nerves and muscles alters the temperature and local humidity, with the possibility of altering the final result of the electroneuromyography. The results corroborated part of this affirmation since the animals in the Normal group presented a significantly higher temperature when compared to the Surgical group. However, there was no statistical difference between the values for latency, amplitude, area, and duration of the muscular response to the electrical stimulation. According to Stecker & Baylor,[16] in a study of the effect of temperature on the nerve action potential, there were alterations in the amplitude and area with temperatures lower than 27oC, and permanent loss of the nerve action potential appeared only after cooling below 100C for long periods. Thus, the result shows that the decrease in body temperature (from 34.46oC to 33.76oC) by the surgical exposure of nerves and muscles, in this experimental model, was not sufficient to cause alteration in the electrophysiological parameters, which corroborates the viability of the procedure.

Greater stimulation was necessary to trigger a potential for muscular action in the Normal group (47.1 ± 9.3 volts) when compared to the Surgical group (4.8 ± 0.6 volts). This difference is due to the decrease in resistance caused by the underlying tissues between the skin and the target nerve in the normal group, nonexistent in the surgical group where the stimulation was applied directly to the nerve.

In addition to the results demonstrated, it is noteworthy that the surgical exposure of nerves and muscles of lower limbs propitiated the stimulation of an exact point on the target nerve. This possibility is important in studies about nervous regeneration or degeneration, where proximal and/or distal stimuli to the lesions or to the surgical repairs are necessary.

CONCLUSION

The diminution of corporal temperature in the rats submitted to surgical exposure of nerves and muscles was not sufficient to alter the values of the electro-physiological parameters observed, validating the procedure in experimental analyses. In addition, the surgical exposure of nerves and muscles allows stimulation of an exact point on the target nerve facilitating the electro-physiological test in studies about nervous regeneration or degeneration.

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